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# **Anthraquinone Purity Issues**

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# Purity Reporting Guidelines

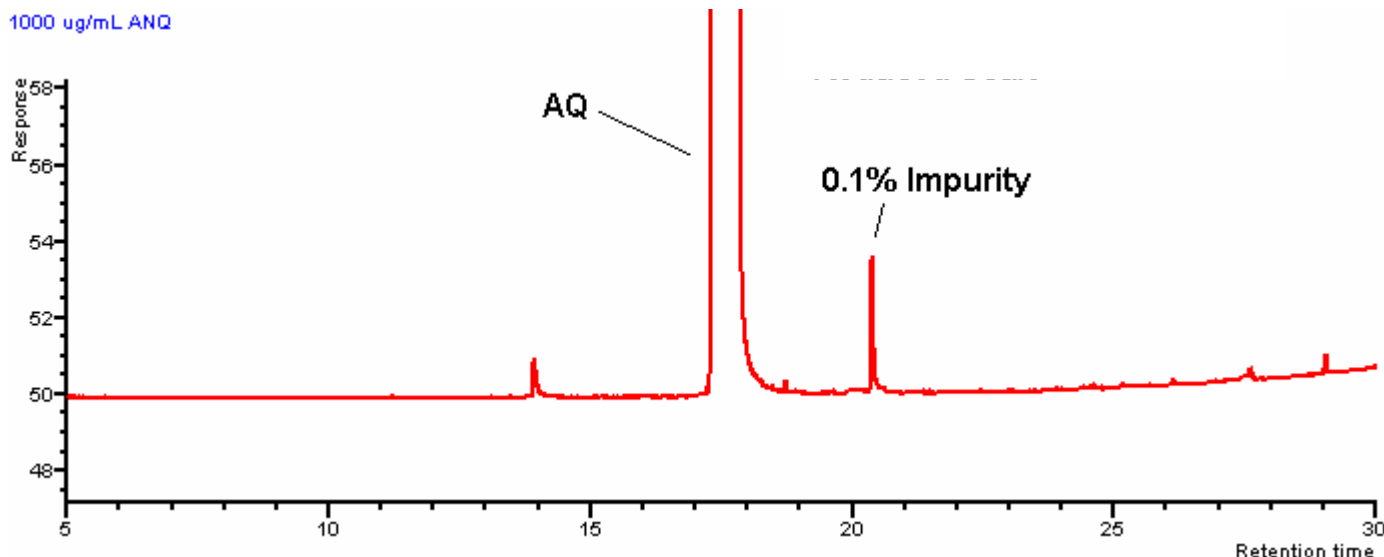
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**The program paradigm for purity analysis in a test article to be used in ETP studies is:**

Present at 1 % or greater relative to the main component = identity characterization

Present at less than 1 %, but is present at 0.1 % or greater relative to the main component = report

# Anthraquinone Purity Analysis by GC/FID



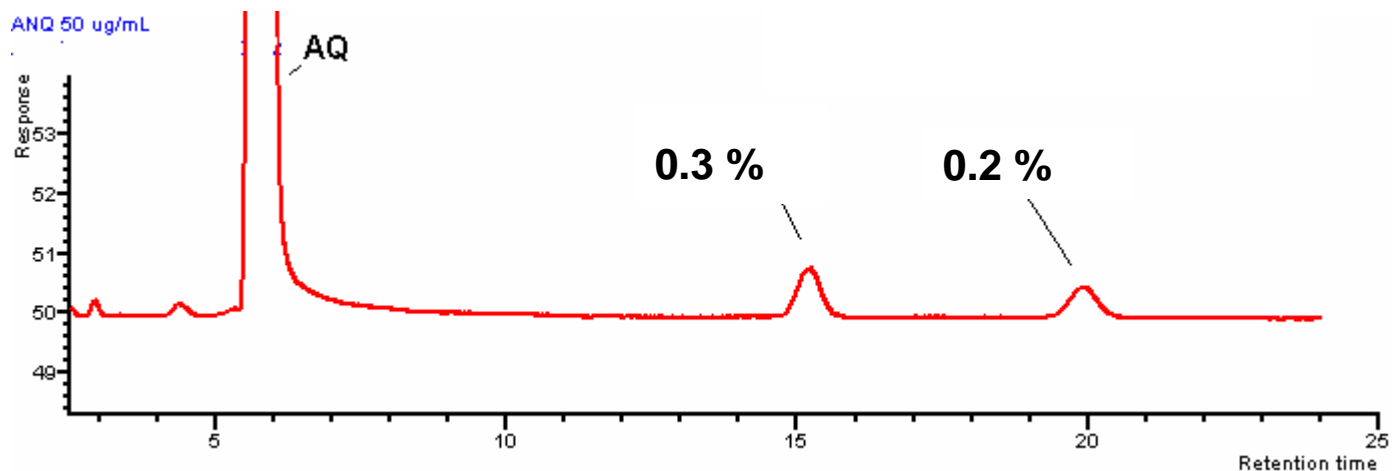
**Initial analysis by GC/FID - 0.1 % impurity**

**Others – much smaller and therefore not reported**

**Overall purity estimate = 99.9 %**

# Anthraquinone Purity Analysis by HPLC/UV

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**Initial analysis by HPLC/UV – reported impurities were 0.2 and 0.3 % of total peak area**

**Others smaller and not reported**

**Overall purity estimate = 99.5 %**

# Impurity Questions

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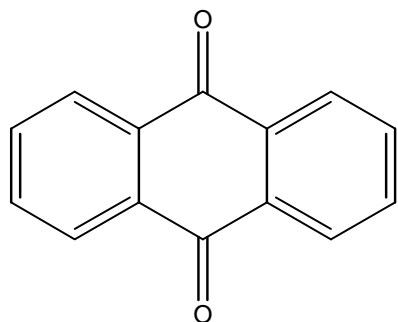
Subsequent analytical chemistry efforts focused on the following :

- 1) Establish unequivocal identities of impurities
- 2) Reconcile the GC/FID and HPLC/UV purity values
- 3) Quantitate impurities against authentic standards

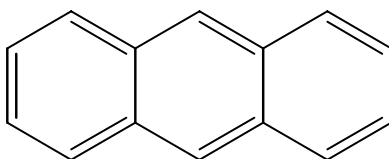
# Impurity Questions 1) and 2)

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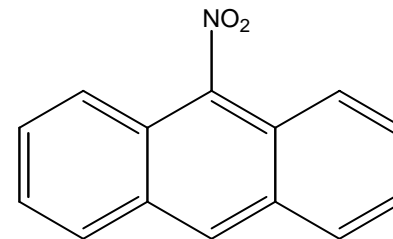
1) 5 compounds could be identified using mass spectrometry and retention time matching:



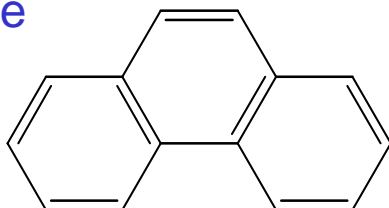
Anthraquinone



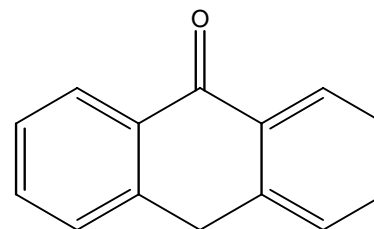
Anthracene



9-Nitroanthracene



Phenanthrene

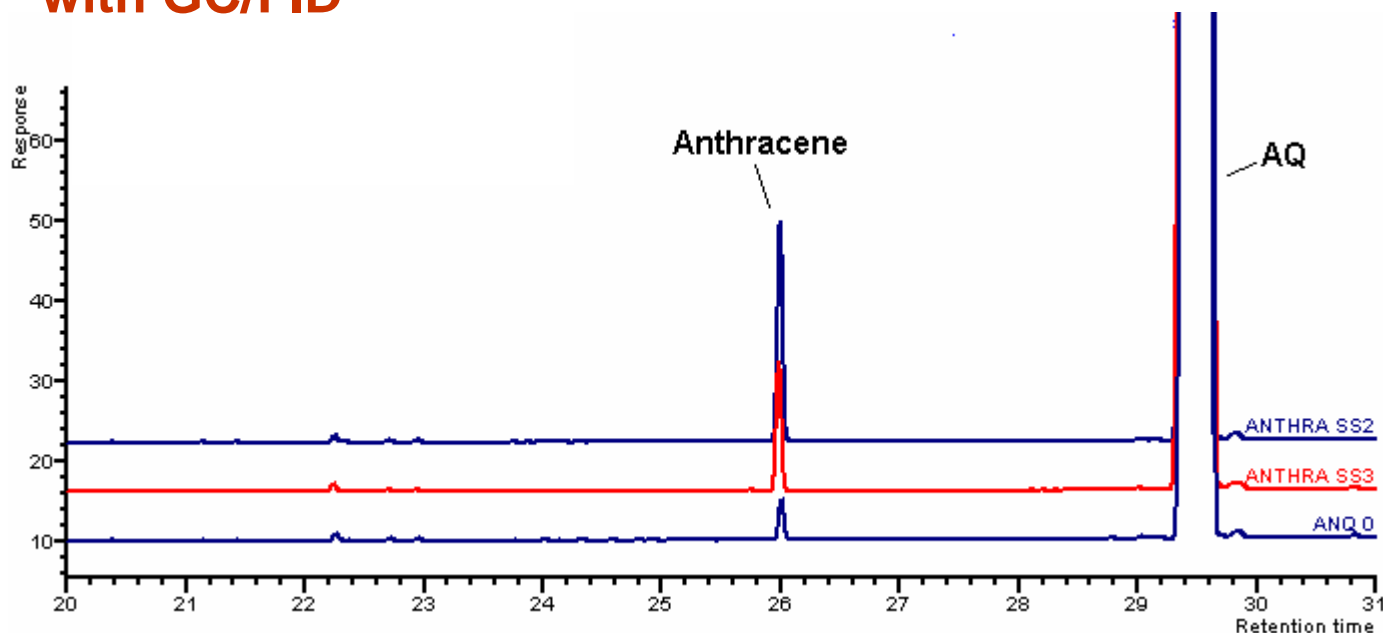


Anthrone

2) Ultraviolet absorbance roughly doubles with each conjugated double bond

# Impurity Question 3)

## Anthracene Impurity Quantitation by Standard Addition with GC/FID



Anthracene = 0.05 %

9-Nitroanthracene = 0.1 %

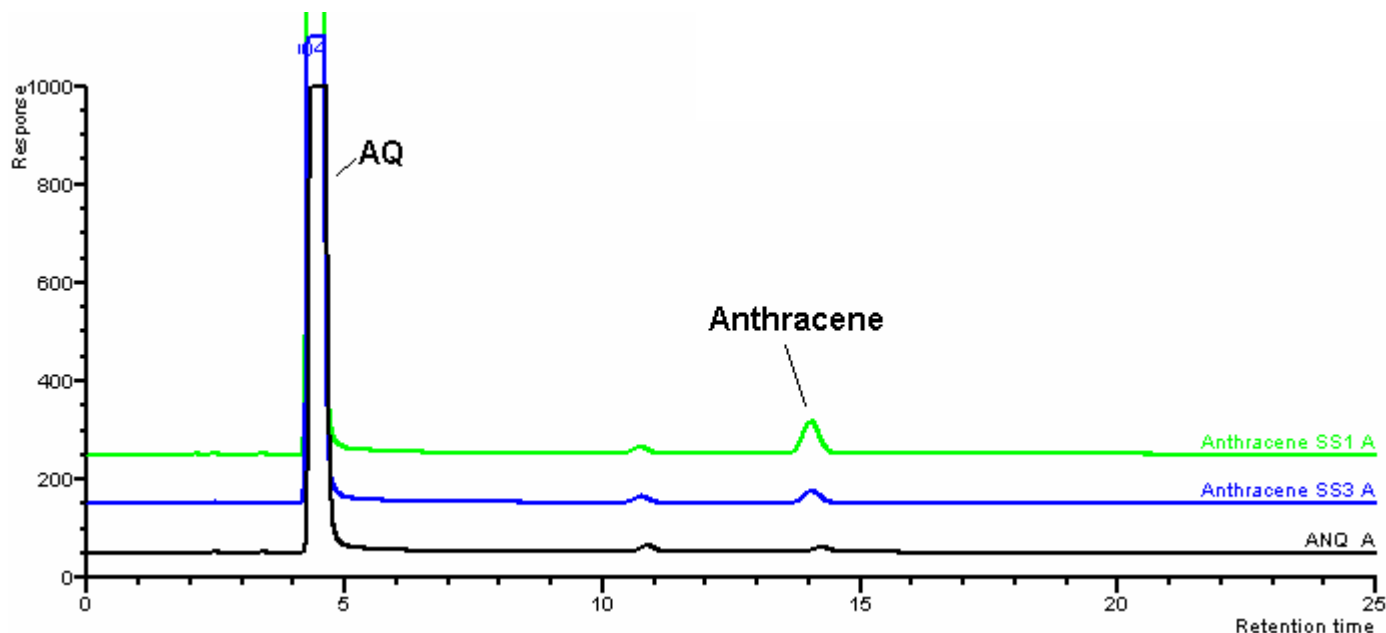
Phenanthrene = < 0.002 %

Anthrone = < 0.008 %

Overall purity = 99.85 %

# Impurity Question 3) cont'd

## Quantitation of Anthracene Impurity by Standard Addition with HPLC/UV



Anthracene = 0.06 %

9-Nitroanthracene = 0.11 %

Phenanthrene = < 0.001%

Overall purity = 99.83 %

# Resolution of Impurity Questions

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- 1) Impurity identifications – 9-nitroanthracene, anthracene, phenanthrene, and anthrone could be unequivocally identified by MS and RT matching
- 2) Difference between GC purity and HPLC purity – this is due to the difference in the relative strength of the UV chromophores in these compounds
- 3) Quantitating impurities against authentic standards – purity is greater than 99.8 %